Appln. No.: 10/672,735
Amendment Date: December 20, 2006
Reply to Office Action of October 20, 2006

Amendments to the Claims: This listing of claims will replace all prior versions, and listings, of claims in the application

Listing of Claims:

1. (Currently Amended) A method for producing a stabilized double D loop at a target sequence within a double-stranded nucleic acid, the method comprising:

providing a first oligonucleotide and a second oligonucleotide, said first and second oligonucleotides having at least a region of complementarity therebetween;

wherein said first oligonucleotide is bound by a recombinase and has a region that is substantially complementary in sequence to a first strand of said target, and said second oligonucleotide is free of a recombinase and has a region that is substantially complementary in sequence to a second strand of said target;

combining said first oligonucleotide ~~and second oligonucleotides~~ with said double-stranded nucleic acid under conditions suitable for production of a double D-loop to form a mixture; and

adding the second oligonucleotide to the mixture, producing said stabilized double D loop at said target sequence within said double-stranded nucleic acid.

2. (Cancelled)

3. (Original) The method of claim 1, wherein said second oligonucleotide comprises at least one modification selected from the group consisting of: locked nucleic acid (LNA) monomer, 2'-OMe monomer, peptide nucleic acid, and phosphorothioate linkage.

4. (Previously Presented) The method of claim 1, wherein said recombinase is *E. coli* RecA protein.

5. (Original) The method of claim 1, further comprising the subsequent step of deproteinizing said double-stranded nucleic acid.

6. (Original) A double-stranded nucleic acid having a stabilized double D loop formed by the method of claim 1.

7. (Currently Amended) A method for detecting the presence of a desired target sequence within a double-stranded nucleic acid, the method comprising:

providing a first oligonucleotide and a second oligonucleotide; wherein said first oligonucleotide is bound by a recombinase and has a region that is substantially complementary in sequence to a first strand of said target, and said second oligonucleotide is free of a recombinase and is substantially complementary in sequence to a second strand of said desired target, and said first oligonucleotide and second oligonucleotide have at least a region of complementarity therebetween;

combining said first oligonucleotide ~~and second oligonucleotides~~ with said double-stranded nucleic acid under conditions suitable for production of a double D-loop to form a mixture;

adding the second oligonucleotide to the mixture; and

detecting stabilized double D-loops having said oligonucleotides, said stable double D-loops signaling the presence of a desired target sequence.

8. (Cancelled)

9. (Original) The method of claim 7, wherein said second oligonucleotide comprises at least one modification selected from the group consisting of: locked nucleic acid (LNA) monomer, 2'-OMe monomer, peptide nucleic acid, and phosphorothioate linkage.

10. (Original) The method of claim 7, wherein at least one of said oligonucleotides is detectably labeled.

11. (Currently Amended) The method of claim 7, further comprising the step, after said combining and adding ~~contacting~~ and before detecting, of: deproteinizing said nucleic acid sample.

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12. (Currently Amended) A method for detecting the presence of a desired target sequence in a sample of double-stranded nucleic acids suspected of having sequences that differ at a target therein, the method comprising:

contacting said sample of double-stranded nucleic acids with a first oligonucleotide to form a mixture and ~~a second oligonucleotide~~,

adding a second oligonucleotide to the mixture, wherein said first oligonucleotide is bound by a recombinase, said second oligonucleotide is free of a recombinase, and said first and second oligonucleotides have at least a region of complementarity therebetween,

wherein both of said first and said second oligonucleotides have regions that are perfectly complementary to respective first and second strands of said desired target sequence, but at least one of said oligonucleotides is imperfectly matched in said region to each of said target sequences that differ from said desired sequence;

deproteinizing said nucleic acids; and ~~then~~

detecting stable double D-loops, said stable double D-loops signaling the presence of a desired target sequence.

13. (Cancelled)

14. (Original) The method of claim 12, wherein said second oligonucleotide comprises at least one modification selected from the group consisting of: locked nucleic acid (LNA) monomer, 2'-OMe monomer, peptide nucleic acid, and phosphorothioate linkage.

15. (Currently Amended) A method for detecting, in a sample of double-stranded nucleic acids suspected of having sequences that differ at a target, the presence of at least two different target sequences, the method comprising:

forming double D-loops at said target by mixing a ~~using a mixture of~~ first oligonucleotide species with the sample of double-stranded nucleic acids to form a mixture ~~and at least one species of second oligonucleotide~~, wherein said mixture includes at least two species of first oligonucleotide, each of said species having a region that is perfectly

complementary to a distinct one of said different target sequences, and each of said species is bound by a recombinase;

adding at least one species of a second oligonucleotide, wherein each of said at least one second oligonucleotide species is free of recombinase; and

wherein said first oligonucleotides and said second oligonucleotides have at least a region of complementarity therebetween;

deproteinizing said nucleic acids; and then

discriminably detecting the species of first oligonucleotides present among stable D-loops, and thereafter determining the presence of at least two different target sequences.

16. (Original) The method of claim 15, wherein each of said first oligonucleotide species is discriminably labeled.

17. (Original) The method of claim 16, wherein each said first oligonucleotide species is labeled with a different fluorophore, said fluorophores having distinguishable emission spectra.

18. (Original) The method of claim 15, wherein said double-stranded nucleic acids are selected from the group consisting of: linear nucleic acids, relaxed closed circular DNA, supercoiled circular DNA, artificial chromosomes, BACs, YACs, nuclear chromosomal DNA, and organelle chromosomal DNA.

19. (Original) The method of claim 15, wherein said second oligonucleotide comprises at least one modification selected from the group consisting of: locked nucleic acid (LNA) monomer, 2'-O-Me monomer, peptide nucleic acid, and phosphorothioate linkage.

20. (Original) The method of claim 15, further comprising the step, after said deproteinizing and before said discriminably detecting, of: separating double D loop-containing nucleic acids from double-stranded nucleic acids lacking double D loops.

21. (Original) The method of claim 20, wherein said first oligonucleotide species, or said second oligonucleotide species, or both said first and second oligonucleotide species comprises a capture moiety, and said separating step is performed by specific binding to said capture moiety.

22. (Currently Amended) A method of purifying, from a mixture of double-stranded nucleic acids having sequences that differ at a target therein, double-stranded nucleic acids having a desired target sequence, the method comprising: forming double D loops at said target ~~using~~ by mixing a first oligonucleotide with the mixture of double-stranded nucleic acids ~~and a second oligonucleotide~~, wherein said first oligonucleotide is bound by a recombinase, adding a ~~said~~ second oligonucleotide, wherein the second oligonucleotide is free of ~~is not substantially bound by said~~ recombinase, and said first and second oligonucleotides have at least a region of complementarity therebetween, wherein said first oligonucleotide is perfectly complementary to a first strand of said desired target sequence, said second oligonucleotide is perfectly complementary to a second strand of said desired target sequence, and at least one of said oligonucleotides is imperfectly matched at each of said target sequences that differ from said desired target sequence; and then purifying double-stranded nucleic acids having stable D loops.

23. (Original) The method of claim 22, wherein said step of forming double D loops comprises: contacting said mixture of double-stranded nucleic acids first with said first, recombinase-bound oligonucleotide and thereafter with said second, recombinase-free oligonucleotide.

24. (Original) The method of claim 22, further comprising the step, after forming double D loops and before purifying, of deproteinizing said double-stranded nucleic acids.

25. (Original) The method of claim 22, wherein said second oligonucleotide comprises at least one modification selected from the group consisting of: locked nucleic acid (LNA) monomer, 2'-O-Me monomer, peptide nucleic acid, and phosphorothioate linkage.

26. (Original) The method of claim 22, wherein said first oligonucleotide, said second oligonucleotide, or both said first and second oligonucleotides comprises a capture moiety, and said purifying step is performed by specific binding to said capture moiety.

27. (Currently Amended) A method of protecting a restriction site target within double-stranded nucleic acids from cleavage during a restriction digest, comprising:

forming double D-loops at said target ~~using~~ by mixing a first oligonucleotide with the double-stranded nucleic acids to form a mixture ~~and a second oligonucleotide~~, wherein said first oligonucleotide is bound by a recombinase and has at least a region that is substantially complementary in sequence to a first strand of said target;

adding a second oligonucleotide to the mixture, wherein said second oligonucleotide is free of recombinase and has at least a region that is substantially complementary in sequence to a second strand of said target; and

wherein said double D-loop is resistant to restriction cleavage at said target;

digesting said double-stranded nucleic acids with a restriction enzyme that recognizes said target sequence; and

detecting resistance of said target sequence to restriction cleavage.

28. (Cancelled)

29. (Original) The method of claim 27, wherein either or both of said oligonucleotides are methylated and said restriction enzyme target site is unmethylated.

30. (Original) The method of claim 27, wherein each of said oligonucleotides contains a mismatch to its respective target sequence strand.

31. (Original) The method of claim 27, wherein said second oligonucleotide comprises at least one modification selected from the group consisting of: locked nucleic acid (LNA) monomer, 2'-O-Me monomer, peptide nucleic acid, and phosphorothioate linkage.

32. (Original) The method of claim 27, further comprising the step, after said forming of D loops and before digestion, of: deproteinizing said nucleic acids.

33. (Currently Amended) A method of cleaving at or near a target sequence within a double-stranded nucleic acid, the method comprising:

forming a double D-loop at said target ~~using~~ by mixing a first oligonucleotide ~~and a second oligonucleotide~~ with the double-stranded nucleic acid to form a mixture, wherein said first oligonucleotide has at least a region that is substantially complementary in sequence to a first strand of said target and is bound by a recombinase;

adding a second oligonucleotide to the mixture, wherein said second oligonucleotide has at least a region that is substantially complementary in sequence to a second strand of said target and is free of recombinase;

reacting said double-stranded nucleic acid with an enzyme that cleaves the double-stranded nucleic acid; and

detecting cleavage at or near said target sequence.

34. (Original) The method of claim 33, wherein said first oligonucleotide:first target strand duplex and/or said second oligonucleotide:second target strand duplex of said D loop forms a type IIs, type IIs-like, or type IIB restriction enzyme site in said double-stranded nucleic acid.

35. (Currently Amended) A method ~~of~~ cleaving at or near a target sequence within a double-stranded nucleic acid, the method comprising:

forming a double D-loop at said target ~~using~~ by making a first oligonucleotide ~~and a second oligonucleotide~~, with the double-stranded nucleic acid to form a mixture, wherein said first oligonucleotide is bound by a recombinase and has at least a region that is substantially complementary in sequence to a first strand of said target;

adding a second oligonucleotideto the mixture, wherein said second oligonucleotide is free of recombinase and has at least a region that is substantially complementary in sequence to a second strand of said target;

reacting said double-stranded nucleic acid with an enzyme that cleaves at or near said double D-loop; and

detecting cleavage at or near said target sequence.

36. (Withdrawn) A kit for forming stabilized double D loops at a target sequence in double-stranded nucleic acids, comprising: a first composition comprising a first oligonucleotide, said first oligonucleotide being bound by a recombinase and having a region that is substantially complementary in sequence to a first strand of said target; and a second composition comprising a second oligonucleotide, said second oligonucleotide being not substantially bound by a recombinase and having a region that is substantially complementary in sequence to a second strand of said target.

37. (Withdrawn) The kit of claim 36, wherein said second oligonucleotide comprises at least one modification selected from the group consisting of: locked nucleic acid (LNA) monomer, 2'-OM e monomer, peptide nucleic acid, and phosphorothioate linkage.

38. (Withdrawn) The kit of claim 36, wherein at least one of said oligonucleotides is detectably labeled.

39. (Withdrawn) The kit of claim 36, wherein at least one of said oligonucleotides is conjugated to a capture moiety.

40. (Withdrawn) A kit for detecting the presence of at least two different target sequences in a sample of double-stranded nucleic acids suspected of having sequences that differ at a target therein, comprising: a composition comprising (i) a mixture of first oligonucleotide species and (ii) at least a first species of second oligonucleotide, wherein said mixture includes at least two species of first oligonucleotides, each of said species having a region that is perfectly complementary to a distinct one of said differing target sequences, and each of said first oligonucleotide species in said mixture being bound by a recombinase; wherein each of said at least one second oligonucleotide species is not substantially bound by said recombinase; and wherein each of said first oligonucleotide species in said mixture and said at least one second oligonucleotide has at least a region of complementarity therebetween.

41. (Withdrawn) The kit of claim 40, further comprising: instructions suitable for performing the method of claim 15.